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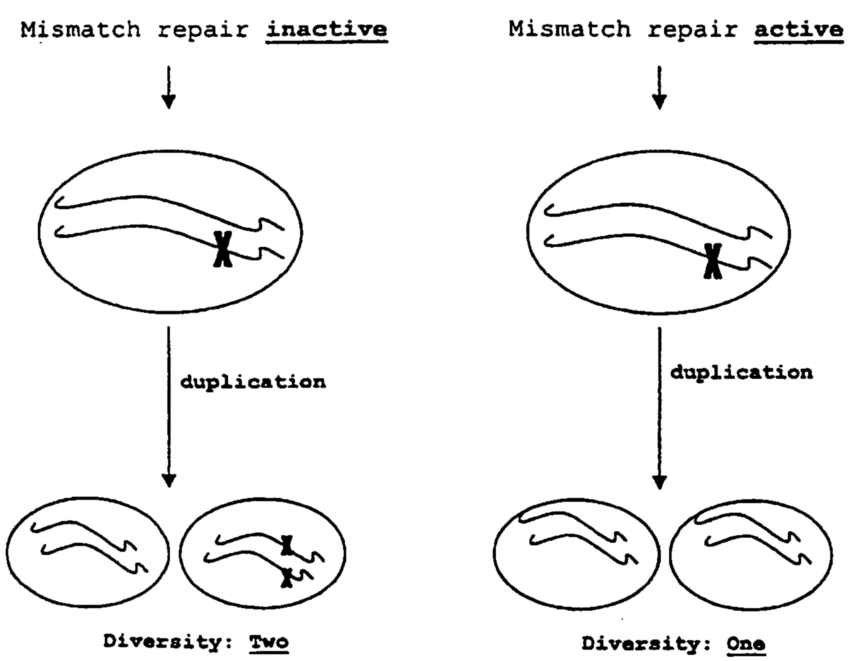

(57) Abstract

A process for making DNA libraries in filamentous fungal cells using a novel cloned gene involved in the mismatch repair system of filamentous fungal cells.

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FUNGAL CELLS WITH INACTIVATED DNA MISMATCH REPAIR SYSTEM

FIELD OF INVENTION

A process for making DNA libraries in filamentous fungal cells using a novel cloned gene involved in the mismatch repair system of filamentous fungal cells.

BACKGROUND OF THE INVENTION

The mismatch repair system is a system within cells which recognises mismatches in newly synthesised duplex DNA sequences.

The mismatch repair system then either corrects the mismatches which are seen as errors by *e.g.* using the methylated ``old'' strain as template or alternatively it may mediate degradation of the duplex DNA sequences which comprise the mismatches.

Independently on the precise mechanism the end result will be that the ``mismatch repair system'' will limit the ``diversity'' within a cell, diversity being represented as duplex DNA sequences which comprise mismatches.

For example a duplex DNA sequence which comprises a single mismatch represents a diversity of two different DNA sequences within the cell. If the mismatch repair system corrects the mismatch there will only be a diversity of one within the cell.

Alternatively, if the mismatch repair system mediates the degradation of such a duplex DNA sequence the diversity will be lost. See figure 1 for a graphic illustration on how the mismatch repair system may work within a cell.

Consequently, if duplex DNA sequences comprising mismatches represent a DNA library of interest, then the diversity of this library may be limited when transformed (placed) into cells with an active mismatch repair system.

The art provides a solution to this problem by making cells wherein the mismatch system is inactive.

EP 449923 describes bacterial cells wherein the mismatch system is inactivated.

WO 97/37011 describes yeast cells wherein the mismatch system is inactivated. See the working examples of this document.

WO 97/05268 describes mice cells wherein the mismatch system is inactivated. See the working examples of this document.

SUMMARY OF THE INVENTION:

The problem to be solved by the present invention is to provide an improved strategy for making DNA libraries in filamentous fungal cells. A filamentous fungal cell population comprising such a DNA library may then be used to select a polypeptide of interest. Also polynucleotide sequences with particular properties might be selected, such as promoters, terminators and other regulatory elements with changed/improved properties.

The solution is based on that the present inventors have cloned a NOVEL gene involved in the mismatch repair system of a filamentous fungal cell. Further, this gene is the first gene cloned which is involved in the mismatch repair system of a filamentous fungal cell.

By inactivating this gene in a filamentous cell it is possible to obtain a filamentous cell which is deficient in its mismatch repair system and which is highly useful for preparing DNA libraries in filamentous fungal cells.

The gene comprises a very characterising DNA sequence encoding the polypeptide sequence shown in positions 683-758 of SEQ ID NO 2.

This DNA has been used to clone the full length gene encoding the polypeptide sequence shown in positions 1-940 of SEQ ID NO 2. See working examples herein (vide infra).

The gene cloned as described in working examples herein is a gene cloned from an *Aspergillus oryzae* filamentous fungal cell.

However, based on the novel sequence information provided herein it is routine work for the skilled person to clone

similar homologous genes from other filamentous fungal cells by e.g. standard hybridisation or PCR technology, preferably by using the DNA sequence encoding the polypeptide sequence shown in positions 683-758 of SEQ ID NO 2 as a basis for making a hybridisation probe or PCR primers.

Accordingly, in a first aspect the present invention relates to a filamentous fungal cell, wherein a gene involved in the mismatch repair system has been inactivated and in which the gene involved in the mismatch repair system comprises:

(a) a DNA sequence encoding the polypeptide sequence shown in positions 683-758 of SEQ ID NO 2; or
(b) a DNA sequence encoding a polypeptide sequence which is at least 70% identical to the polypeptide sequence shown in positions 683-758 of SEQ ID NO 2;

and

in a second aspect the present invention relates to a filamentous fungal cell, wherein a gene involved in the mismatch repair system has been inactivated and in which the gene involved in the mismatch repair system comprises:

(a) a DNA sequence encoding the polypeptide sequence shown in positions 1-940 of SEQ ID NO 2; or
(b) a DNA sequence encoding a polypeptide sequence which is at least 70% identical to the polypeptide sequence shown in positions 1-940 of SEQ ID NO 2.

As stated above a filamentous fungal cell of the first or second aspect of the invention is very suitable for making a DNA library of interest in filamentous fungal cells.

Accordingly, in a third aspect the present invention relates to a process for preparing a filamentous fungal cell population wherein individual cells in the population comprise individually different DNA sequences of interest representing a DNA library of interest comprising following steps:

(a) placing individually different DNA sequences of interest in a filamentous fungal cell population comprising a filamentous fungal cell of the first or second aspect of the invention; and
(b) growing the population of (a) for a period of time allowing an individual DNA sequence of interest in the

population to be duplicated at least once under conditions wherein the mismatch repair system gene of the first or second aspect of the invention has been inactivated.

One of the advantages of allowing an individual mismatch repair inactivated filamentous fungal cell duplicated DNA of interest at least once as descried under step (b) of the third aspect is illustrated in figure 1. As can be seen in figure 1 the process of the third aspect using a filamentous fungal mismatch repair inactivated cell as described herein allows preparation of a DNA library wherein eventual hetero duplex DNA mismatches are not corrected. This gives a DNA library with a higher diversity as compared to a DNA library made in a filamentous fungal cell NOT having an inactivated mismatch repair system (see figure 1). Duplication of DNA sequence of interest means that the two strands are replicated such that two separate sets of double stranded DNA are generated, each being based on a separate one of the two original strands.

A filamentous fungal cell population wherein individual cells in the population comprise a DNA library of interest as described above may be used to select a polypeptide of interest.

Accordingly, in a fourth aspect the present invention relates to a process for production of a polypeptide of interest comprising the steps of the third aspect and wherein the DNA sequences of interest encode a polypeptide of interest and which further comprises following step:

(c) selecting from the resultant population of filamentous fungal cells of step (b) of the third aspect a desired polypeptide of interest.

An advantage of the process of the fourth aspect may be that the polypeptide of interest is selected from a filamentous fungal cell expressing the polypeptide. Consequently, it is directly known that the polypeptide can be expressed from a filamentous fungal cell, which is usefully if it is subsequently required to produce the polypeptide in large scale in a filamentous fungal cell. This may be of particular

interest when the DNA library encodes polypeptides of interest which are derived from filamentous fungal cells, since it is known that filamentous fungal polypeptides preferably are produced in industrial relevant high yields in filamentous fungal cells.

This is contrary to a similar selection process using e.g. a yeast cell. Here the only thing known is that the selected polypeptide is capable of being expressed in yeast and later expression a filamentous fungal cell might give problems, especially if high yields are required.

DEFINITIONS:

Following section provides definitions of technical features in above-mentioned aspects of the invention.

The term ``a gene'' denotes herein a gene (a DNA sequence) will is capable of being expressed into a polypeptide within said cell. Accordingly, said gene sequence will be defined as an open reading frame starting from a start codon (normally ``ATG'', ``GTG'', or ``TTG'') and ending at a stop codon (normally ``TAA'', TAG'' or ``TGA'').

In order to express said gene there must be elements, as known in the art, in connection with the gene, necessary for expression of the gene within the cell. Such standard elements may include a promoter, a ribosomal binding site, a termination sequence, and may be others elements as known in the art.

The term ``mismatch repair system'' shall herein be understood according to the art, as a system within cells which recognises mismatches in duplex DNA sequences. See e.g. WO 97/37011, page 1, line 21-28)

The mismatch repair system then either corrects the mismatches which are seen as errors by *e.g.* using the methylated ``old'' strain as template or alternatively it may mediate degradation of the duplex DNA sequences which comprise the mismatches.

Independently on the precise mechanism the end result will be that the ``mismatch repair system'' will limit the ``diversity'' within the cell represented by such duplex DNA sequences which comprise mismatches.

For example a duplex DNA sequence which comprises a single mismatch represents a diversity of two different DNA sequences within the cell. If the mismatch repair system corrects the mismatch their will only be a diversity of one within the cell. Alternatively, if the mismatch repair system mediates the degradation of such a duplex DNA sequence this diversity will be lost.

A polypeptide encoded by a gene involved in the mismatch repair system recognises a mismatch by a mechanism involving binding to the mismatch.

Accordingly, a suitable assay to test whether or not a filamentous fungal cell as described herein is inactivated in its mismatch repair system is to use a ``gel shift assay'' or alternatively termed a ``gel retardation assay''. This is a standard assay used in the art. See WO 97/05268, page 16,17 and 25.

The principle in such an assay is that cell extracts are prepared of both (a) a filamentous fungal cell wherein the gene, as described herein, involved in the mismatch repair system is inactivated; and (b) the corresponding filamentous fungal cell wherein the gene is NOT inactivated. These extracts are then bound/mixed to oligonucleotides containing the base-pair mismatched G:T; G:A; G:G; A:C, and an extrahelical TG dinucleotide and run on a nondenaturing gel.

If the gel shift assay demonstrates that the control filamentous fungal cell wherein the gene is NOT inactivated comprises any protein(s) which binds to any of above mentioned oligonucleotides and these binding protein(s) are NOT seen in the filamentous fungal cell wherein the gene, as described herein, involved in the mismatch repair system is inactivated then it is a confirmation that the mismatch repair system in the latter is inactivated.

A detailed description of a suitable gel shift assay is provided in working example 1 herein.

The sequence identity in relation to the terms

``<u>a DNA sequence encoding a polypeptide sequence which is at least *70% identical* to the polypeptide sequence shown in positions 683-758 of SEQ ID NO 2</u>'' and

"<u>a DNA sequence encoding a polypeptide sequence which is at least *70% identical* to the polypeptide sequence shown in positions 1-940 of SEQ ID NO 2</u>";

is determined as the degree of identity between two sequences indicating a derivation of the first sequence from the second. The identity may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453). Using GAP with the following settings for polypeptide sequence comparison: GAP creation penalty of 3.0 and GAP extension penalty of 0.1, the polypeptide encoded by an analogous DNA sequence of the invention exhibits a degree of identity preferably of at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably at least 95%, and especially at least 97% with amino acid sequence shown in positions 683-758 of SEQ ID NO 2, according to the first aspect of the invention; or with amino acid sequence shown in positions 1-940 of SEQ ID NO 2, according to the second aspect of the invention.

The term "<u>DNA library</u>" denotes herein a library of at least two different DNA sequences. For many practical purposes the library is must bigger. Accordingly, the DNA library preferably comprises at least 1000 different DNA sequences, more preferably at least 10000 different DNA sequences, and even more preferably at least 100000 different DNA sequences.

The term "<u>placing individually different DNA sequences of interest in a filamentous fungal cell population</u>" in relation to step (a) in the process of the third aspect of the invention shall herein be understood broadly in the sense that it is NOT identical DNA sequences of interest which are placed in the filamentous fungal cell population. In the present context, relating to a process for making a DNA library using a mismatch repair deficient cell, the term should preferably denotes a situation wherein a cell within the filamentous fungal cell population comprises at least two different DNA sequences of

interest which are so partially homologous that they are capable of hybridising/recombining to each other within the cell. It is within the skilled persons general knowledge to determine how partially homologous such sequences have to be in order to obtain said recombination within the cell.

A practical example may be that single stranded oligonucleotide sequences partially homologous to chromosomal DNA sequence are placed within the cell or duplex DNA sequences comprising mismatches (e.g. comprised within a vector) are placed within the cell. See below for further description of such examples.

The specific experimental way of placing these DNA sequences within a filamentous cell may be done according to any of the many suitable techniques, such as transformation techniques.

The term ``growing the population of (a) for a period of time allowing an individual DNA sequence of interest in the population to be *duplicated at least once* under conditions wherein the mismatch repair system gene has been inactivated'' according to step (b) of the third aspect of the invention denotes that after an individual cell has duplicated itself at least once then the mismatch repair system may be activated again without loosing the advantage of the process. The technical reason for this is illustrated in figure 1. In this example a duplex DNA sequence comprising a single mismatch is placed in filamentous cell. After the cell has been duplicated once under conditions wherein the mismatch repair system gene has been inactivated the two individually different single stranded DNA sequences within the duplex DNA have individually been duplicated providing two different duplex sequences, one in each duplicated cell, without any mismatches. Accordingly, since such a cell does NOT comprise duplex DNA sequences of interest having mismatches then there is no technical need to maintaining the mismatch repair system inactivated.

In sections below are described preferred embodiments of the invention by way of examples only.

DRAWINGS:

Figure 1:

This figure illustrates an example wherein a duplex DNA sequence comprising a single mismatch is placed in filamentous cell. After the cell has been duplicated once under conditions wherein the mismatch repair system gene has been inactivated the two individually different single stranded DNA sequences within the duplex DNA have individually been duplicated providing two different duplex sequences, one in each duplicated cell, without any mismatches. On the contrary, in a cell wherein the mismatch repair system is active, a mismatch within a duplex is corrected.

Figure 2:

This figure shows three partial *Aspergillus oryzae* polypeptide sequences: `msh2'Ao-col10/13/15; derived from cloned PCR fragments. The three partial polypeptide sequences are aligned with two other partial polypeptide sequences of known mismatch repair proteins: a human mismatch repair protein, msh2-human.p; and a fungal *Saccharomyces cerevisiae* mismatch repair protein, S.c. msh2. The underlined sequences in the figure derive from the construction of the PCR fragments.

Figure 3:

This figure shows an alignment of the proposed polypeptide sequence of the putative *Aspergillus oryzae* mismatch repair protein (Ao.MSH2) with the polypeptide sequences of three known mismatch repair proteins from human (msh2-human.p), mouse (msh2-mus.p), and yeast (S.c. msh2).

DETAILED DESCRIPTION OF THE INVENTION

A filamentous fungal cell, as described herein, wherein a gene, as described herein, involved in the mismatch repair system has been inactivated.

Inactivation of a gene involved in the mismatch repair system:

The NOVEL gene, as described herein, involved in the mismatch repair system may be inactivated by any of the numerous known techniques known to the skilled person.

An embodiment of the invention relates to a filamentous fungal cell as described herein, wherein the gene involved in the mismatch repair is defective.

Numerous methods are known to the skilled person to make a gene defective when the DNA sequence is KNOWN. These methods includes deleting part of the DNA sequence of the gene; introducing frame-shift mutations by deleting or inserting nucleotides; introducing stop codons etc.

A preferred embodiment of the invention relates to a filamentous fungal cell as described herein, wherein the gene involved in the mismatch repair has been inactivated transitorily.

Similarly to above, a number of methods are known to the skilled person for doing this, including insertion of a regulable promoter upstream of the gene or e.g. permanently deleting part of the gene on the chromosome followed by inserting a vector (e.g. a plasmid) into the cell which comprises the gene. The plasmid may then comprise a regulable promoter up-steam of the gene or it may be that the plasmid can be removed from the cell when the mismatch repair system shall be inactivated transitorily and then re-inserted into the cell when the mismatch repair system shall be re-activated.

It is within the skilled persons general knowledge to choose the appropriate strategy for a specific technical purpose.

A preferred way to make a filamentous fungal cell which is capable of transitorily inactivate the mismatch repair system as described herein is first to permanently inactive the mismatch repair gene described herein on the chromosome of the cell followed by inserting a plasmid into the cell which comprises the gene, wherein the plasmid is characterised by that it comprises a suitable replication initiating sequence and a suitable selectable marker.

Preferably the suitable replication initiating sequence is AMA1 (Gems, D., et al. (1991, Gene 98:61-67).

A more detailed description of suitable replication initiating sequences and suitable selectable markers is provided immediately below and in working example 4 herein is provided an example of this strategy using a plasmid comprising AMA1 as replication initiating sequence and AmdS as selectable marker.

Replication initiating sequences

As used herein, the term "fungal replication initiating sequence" is defined as a nucleic acid sequence which is capable of supporting autonomous replication of an extrachromosomal molecule, e.g., a plasmid or a DNA vector, in a fungal host cell, normally without structural rearrangement of the plasmid or integration into the host cell genome. The replication initiating sequence may be of any origin as long as it is capable of mediating replication initiating activity in a fungal cell. Preferably, the replication initiating sequence is obtained from a filamentous fungal cell, more preferably a strain of *Aspergillus*, *Fusarium* or *Alternaria*, and even more preferably, a strain of *A. nidulans, A. oryzae, A. niger*, *F. oxysporum* or *Alternaria altenata*.

A replication initiating sequence may be identified by methods well-known in the art. For instance, the sequence may be identified among genomic fragments derived from the organism in question as a sequence capable of sustaining autonomous replication in yeast, (Ballance and Turner, Gene, 36 (1985), 321-331), an indication of a capability of autonomous replication in filamentous fungal cells. The replication initiating activity in fungi of a given sequence may also be determined by transforming fungi with contemplated plasmid replicators and selecting for colonies having an irregular morphology, indicating loss of a sectorial plasmid which in turn would lead to lack of growth on selective medium when selecting for a gene found on the plasmid (Gems et al, Gene, 98 (1991) 61-67). AMA1 was isolated in this way. An alternative way to isolate a replication initiating sequence is to isolate natural occurring

plasmids (e.g. as disclosed by Tsuge et al., Genetics 146 (1997) 111-120 for *Alternaria aternata*).

Examples of replication initiating sequences include, but are not limited to, the ANS1 and AMA1 sequences of *Aspergillus nidulans*, e.g., as described, respectively, by Cullen, D., et al. (1987, Nucleic Acids Res. 15:9163-9175) and Gems, D., et al. (1991, Gene 98:61-67).

The term "replication initiating activity" is used herein in its conventional meaning, i.e. to indicate that the sequence is capable of supporting autonomous replication of an extrachromosomal molecule, such as a plasmid or a DNA vector in a fungal cell.

The term "without structural rearrangement of the plasmid" is used herein to mean that no part of the plasmid is deleted or inserted into another part of the plasmid, nor is any host genomic DNA inserted into the plasmid.

Filamentous fungal selective marker

The term "selective pressure" is defined herein as culturing a filamentous fungal cell, containing a DNA vector containing a fungal selective marker gene operably linked to a polynucleotide sequence of interest, in the presence of an effective amount or the absence of an appropriate selective agent. The effective amount of the selective agent is defined herein as an amount sufficient for allowing the selection of cells containing the selection marker from cells which do not contain the selection marker.

In a preferred embodiment, the fungal selective marker is selected from the group of genes which encodes a product capable of providing resistance to biocide or viral toxicity, resistance to heavy metal toxicity, or prototrophy to auxotrophs.

In a more preferred embodiment, the prototrophy is obtained from an enzyme selected from the group of metabolic pathways consisting of nucleotide synthesis, cofactor synthesis, amino acid synthesis, acetamide metabolism, proline metabolism, sulfate metabolism, and nitrate metabolism.

In an even more preferred embodiment, in the methods of the present invention the fungal selective marker is a gene selected from the group consisting of *argB* (ornithine carbamoyltransferase), *amdS* (acetamidase), *bar* (phosphinothricin acetyltransferase), *hemA* (5-aminolevulinate synthase), *hemB* (porphobilinogen synthase), *hygB* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *prn* (proline permease), *pyrG* (orotidine-5'-phosphate decarboxylase), *pyroA*, *riboB*, *sC* (sulfate adenyltransferase), and *trpC* (anthranilate synthase).

The fungal cell is cultivated in a suitable medium and under suitable conditions for screening or selecting for transformants harbouring the variant polynucleotide sequence of interest having or encoding the desired characteristic. The cultivation may be performed in accordance with methods well-known in the art for screening of polynucleotide variant libraries.

<u>The filamentous fungal cell</u>

The filamentous fungal cell as described herein includes all filamentous forms of the subdivision Eumycota and Oomycota. The filamentous fungi are characterised by a mycelial wall composed of chitin, cellulose, glucan, chitosan, mannan, and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In contrast, vegetative growth by yeasts such as *Saccharomyces cerevisiae* is by budding of a unicellular thallus and carbon catabolism may be fermentative. In a preferred embodiment, the filamentous fungal cell is a cell of a species of, but is not limited to, *Acremonium*, *Aspergillus*, *Fusarium*, *Humicola*, *Mucor*, *Myceliophthora*, *Neurospora*, *Penicillium*, *Scytalidium*, *Thielavia*, *Tolypocladium*, and *Trichoderma*.

Examples of filamentous fungal cells of use in the present invention include an *Aspergillus* cell, an *Acremonium* cell, a *Fusarium* cell, a *Humicola* cell, a *Mucor* cell, a *Myceliophthora* cell, a *Neurospora* cell, a *Penicillium* cell, a *Thielavia* cell, a *Tolypocladium* cell, and a *Trichoderma* cell.

More specifically, the filamentous fungal cell is an *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger*, or *Aspergillus oryzae* cell;

a *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*, *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sporotricioides*, *Fusarium sulphureum*, *Fusarium torulosum*, *Fusarium trichothecioides*, *Fusarium venenatum* cell or a *Fusarium venenatum* cell (Nirenberg sp. nov; a *Humicola insolens* cell or a *Humicola lanuginosa* cell; a *Mucor miehei* cell; a *Myceliophthora thermophila* cell; a *Neurospora crassa* cell; a *Penicillium purpurogenum* cell; a *Thielavia terrestris* cell; or a *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, or *Trichoderma viride* cell.

A process for preparing a filamentous fungal cell population comprising a DNA library according to the third aspect of the invention:

Placing individually different DNA sequences of interest in a filamentous fungal cell population according to step (a) of the process of the third aspect of the invention:

As state above, the specific experimental way of placing these DNA sequences within a filamentous cell may be done according to any of the many suitable techniques, such as transformation techniques. See the general fungal textbook ``Fungal Genetic'' (1996, ISBN 0-8247-9544-X) for a further description of such standard techniques.

A practical example may be that single stranded oligonucleotide sequences partially homologous to chromosomal DNA sequence are placed within the cell. See Calissano et al.

(Fungal genetic newsletter 43:15-16 (1995) for further description of this.

Another example may be that duplex DNA sequences comprising mismatches (e.g. comprised within a vector as shown in figure 1) are placed within the cell.

In an preferred embodiment the different DNA sequences of interest is comprised in a plasmid wherein the plasmid is characterised by that it comprises a suitable replication initiating sequence and a suitable selectable marker as described above.

Preferably the suitable replication initiating sequence is AMA1 (Gems, D., et al. (1991, Gene 98:61-67).

<u>Growing the population of step (a) for a period of time allowing an individual DNA sequence of interest in the population to be duplicated at least once under conditions wherein the mismatch repair system gene, as describe herein has been inactivated, according to step (b) of the third aspect of the invention.</u>

Growing of the population may be done in any of the numerous suitable known media for growing filamentous fungal cells. It is within the skilled persons general knowledge to choose such a suitable media.

As explained above an individual cell in the population must be allowed to be duplicated at least once under conditions wherein the mismatch repair system gene, as described herein has been inactivated.

Said cells may of course be allowed to be *duplicated more than once* under conditions wherein the mismatch repair system gene has been inactivated.

Since inactivation of the mismatch repair system normally will cause accumulation of mutations on the chromosomal DNA within the cell and thereby maybe make lethal mutations to the cell the actual preferred number of duplication cycles as described above will depend on how fast such potential lethal mutations arise.

It is within the skilled persons general knowledge to determine how many of duplication cycles it preferred.

Due to these potential lethal mutations it is preferred that the mismatch repair system under step (b) has been inactivated transitorily.

After suitable cycles of duplication according to step (b) of the third aspect the transitorily inactivated mismatch repair system the mismatch repair system is then re-activated in order to avoid these lethal mutations in the filamentous fungal cell as such. The strategy for this transitorily inactivation may be any of the strategies described above.

Another strategy to limit introduction of mutations on the chromosome, is to transitorily stop the chromosomal replication while replicating the extra-chromosomal element under mismatch repair deficient conditions. This can be achieved by introducing mutations in elements being solely necessary for the chromosomal replication.

A preferred strategy is to use a filamentous fungal cell wherein the gene involved in the mismatch repair system as described herein is permanently inactivated on the chromosome of the cell followed by inserting a plasmid into the cell which comprises the gene, wherein the plasmid is characterised by that it comprises a suitable replication initiating sequence and a suitable selectable marker. See above for a further explanation of this strategy.

Preferably the suitable replication initiating sequence is AMA1 (Gems, D., et al. (1991, Gene 98:61-67).

A further embodiment relates to the process of the third aspect of the invention, wherein the mismatch repair system under step (b) is defective.

In a further embodiment the invention relates to a process as described herein, wherein, under step (b) of the third aspect of the invention, there is an *in vivo* intergenic recombination of partially homologous DNA sequences of interest.

Since the overall concept of the present invention is to provide a process involving inactivation of the mismatch system it is of course preferably that said partially homologous DNA sequences are capable of in vivo forming duplex DNA sequences comprising mismatches.

<u>**A process for production of a polypeptide of interest comprising the steps of the third aspect of the inv ntion and wherein the DNA sequences of interest encode a polypeptide of interest, according to the fourth aspect of the invention:**</u>

<u>Selecting from the resultant population of filamentous fungal cells of step (b) of the third aspect a desired polypeptide of interest, according to step (c) of the fourth aspect.</u>

The desired polypeptide of interest may be any polypeptide comprising an desired technical feature, such as improved stability; a desired specific activity; a desired pH optimum; an improved wash performance in a detergent; etc.

The specific strategy for selecting this desired polypeptide of interest may be any of the numerous selecting strategies known to the skilled person, such as plate screening assays, micro-titer plate based assays, etc.

An embodiment of the invention relates to a process of the fourth aspect of the invention, which further comprises following steps:

(d) an optionally step comprising modifying the amino acid sequence of the desired selected polypeptide of interest according to a particularly further specific need;

(e) placing the DNA sequence encoding the polypeptide of interest of step (c) of the fourth aspect or the modified polypeptide of interest of step (d) into a filamentous fungal cell which is suitable for large scale production of the polypeptide of interest ;

(f) cultivating the filamentous fungal cell of step (e) in a fermentor of at least 10000 m3 under conditions permitting expression of the polypeptide of interest; and

(g) isolating the polypeptide of interest.

This embodiment relates to an industrial very relevant process, wherein the selected polypeptide of interest is produced in large scale.

The optionally step (d) relates to a situation wherein e.g. the desired polypeptide of interest is selected in order to e.g. identify a polypeptide with improved wash performance in a detergent according to step (c) of the third aspect of the invention. This polypeptide while having improved wash performance in a detergent may not be sufficiently stable for a commercial product. Accordingly, it may be required to make some further amino acid substitutions in this selected polypeptide, such as e.g. suitable Proline substitutions on order to obtain sufficient stability to commercialising this polypeptide.

A further embodiment relates to a process of the embodiment immediately above, wherein the filamentous fungal cell which is suitable for large-scale production of the polypeptide of interest of step (e) said embodiment is *another* filamentous fungal cell as compared to the filamentous fungal cell of step (a) of the third aspect of the invention.

This embodiment relates to a situation wherein the filamentous fungal cell used to select the polypeptide of interest is *different* from the one which is used for large scale production.

A further embodiment relates to a process as described herein, wherein the polypeptide of interest is a polypeptide derived from a filamentous fungal cell.

The term derived from a filamentous fungal cell should be understood in the sense that the information in the amino acid sequence of the polypeptide of interest is derived from a polypeptide obtained from a filamentous fungal cell.

Consequently, it may be a variant of a wild-type filamentous fungal polypeptide and/or may be a polypeptide which is a result of a recombination/shuffling of two or more different filamentous fungal polypeptides.

In an even further embodiment the invention relates to a process as described herein, wherein the polypeptide of interest is an enzyme, such as an amylase, a protease, a cellulase, a lipase, a xylanase; a phospholipase.

EXAMPLES:

Materials

Chemicals used as buffers and substrates were commercial products of at least reagent grade.

EXAMPLE 1:

A gel shift assay suitable for determining if a filamentous fungal cell as described herein is inactivated in the mismatch repair system:

The principle in this gel shift assay is that cell extracts are prepared of both (a) a filamentous fungal cell wherein the gene, as described herein, involved in the mismatch repair system is inactivated; and (b) the corresponding filamentous fungal cell wherein the gene is NOT inactivated. These extracts are then bound/mixed to oligonucleotides containing the base-pair mismatched G:T; G:A; G:G; A:C, and an extrahelical TG dinucleotide and run on a nondenaturing gel.

If the gel shift assay demonstrates that the control filamentous fungal cell wherein the gene is NOT inactivated comprises any protein(s) which binds to any of above mentioned oligonucleotides and these binding protein(s) is NOT seen in the filamentous fungal cell wherein the gene, as described herein, involved in the mismatch repair system is inactivated then it is a confirmation that the mismatch repair system in the latter is inactivated.

Experimental procedure:

Preparation of cell extracts are performed as described in Nagata et al. (Mol. Gen Genet (1993) 237:251-260; See Materials and Methods).

Annealing of oligonucleotides, binding of cell extracts to duplex oligonucleotides containing mismatched, and nondenaturing polyacrylamide gelelectrophoresis are performed essentially as described (Stephenson and Karran; Selective binding to DNA base pair mismatches by proteins from human cells; J. Biol. Chem. 264:2177-21182 (1989)).

However, gelelectrophoresis is performed in TAE buffer rather than in TBE buffer. To obtain duplex oligonucleotides,

the oligonucleotide **U** is radiolabelled and annealed with any of the unlabelled oligonucleotides **L-G.T, L-G.A, L-G.C, L-A.C, L-T.G.,** or **L-HOM.** Oligonucleotide sequences are derived from Aquilina et al. Proc. Natl. Acad. Sci. USA 91:8905-8909 (1994).

U: 5´-GGGAAGCTGCCAGGCCCCAGTGTCAGCCTCCTATGCTC-3´ (SEQ ID NO 3);

L-G.T: 5´-GAGCATAGGAGGCTGACATTGGGGCCTGGCAGCTTCCC-3´ (SEQ ID NO 4) (resulting in a G.T mismatch);

L-G.A.: 5´-GAGCATAGGAGGCTGACAATGGGGCCTGGCAGCTTCCC-3´ (SEQ ID NO 5) (resulting in a G.A mismatch);

L-G.G.: 5´-GAGCATAGGAGGCTGACAGTGGGGCCTGGCAGCTTCCC-3´ (SEQ ID NO 6) (resulting in a G.G mismatch);

L-A.C.: 5´-GAGCATAGGAGGCTGACACCGGGGCCTGGCAGCTTCCC-3´ (SEQ ID NO 7) (resulting in a A.C mismatch);

L-TG: 5´-GAGCATAGGAGGCTGACACTGTGGGGCCTGGCAGCTTCCC-3´ (SEQ ID NO 8) (resulting in an extrahelical TG dinucleotide);

L-HOM: 5´-GAGCATAGGAGGCTGACACCGGGGCCTGGCAGCTTCCC-3´ (SEQ ID NO 9) (resulting in a homoduplex).

In all assays, a twofold excess of unlabelled homoduplex competitor oligonucleotide is included.

EXAMPLE 2:

Cloning of a gene involved in the mismatch repair system of an Aspergillus oryzae cell.

The gene cloned as described in this example is shown in SEQ ID N0 1 (DNA sequence) and SEQ ID NO 2 (the translated amino acid sequence).

Several sequences of mismatch repair proteins from various organisms are known, only three of these have been utilized in the following: *S. cerevisiae* (M84170), *H. sapiens* (L47580) and mouse (U21011).

The numbers indicated are reference numbers from the public available GenBank database.

Based on the C-terminal homology between known mismatch repair proteins, a set of degenerate primers were designed, aiming at amplification of a partial sequence of the Aspergillus oryzae homolog:

Pr 117858 (SEQ ID NO 10): P-GGCNCARATHGGNTGYTTYGTNCC
Pr 117859 (SEQ ID NO 11): P-GCCCANGCNARNCCRAANCC

With chromosomal DNA from A. oryzae strain JaL142 (WO 96/29391) as template, and above primers the following 50μl PWO polymerase based PCR reaction was performed at eight $MgSO_4$ concentrations (0.5 mM to 4.0 mM, as recommended by the manufacturer; Boehringer M.). 1 mM $MgSO_4$ was found to be optimal and gave a discrete band of appr. 230 bp as would be expected if no introns were embodied in the sequence.

PCR-cycle profile: [96°C; 2 min - 30 cycles of (94°C; 15s - 50°C; 15s - 72°C; 30s) - 72°C,7 min - 4°C; hold].

The 230bp PCR fragment was blunt end ligated into filled in BamH1 site of pUC19. pUC19 was BamH1 cleaved in presence of calf intestine alkaline phosphatase, followed by filling in the sticky ends by klenow polymerase and dNTP. Three individual plasmids harbouring the insert were isolated from E.coli XL1 transformants of above ligation, and sequenced. Alignment of polypeptides derived by translation of the cloned PCR fragments, revealed a strong homolgy to known mismatch repair protein sequences (see Figure 2).

The underlined sequences of Figure 2 are sequences derived from the consensus PCR primers described above.

The three *Aspergillus* sequences of Figure 2 are equal to the sequence shown in SEQ ID N0 2 from positions 683-758, except from position 685 which in the final cloned sequence is a Thr (T) in sted of an Ile (I) as indicated above. This is due to the sequence in above mentioned consensus primers.

The alignment shown in Figure 2 clearly demonstrates that the cloned fragment originates from an *A. oryzae* homologue of a mismatch repair protein.

In order to clone the entire gene, a radiolabeled probe of the cloned fragment was generated by PCR, using 0.5 mg pUC19'msh2'-13 (see above) as template in a 100 ml reaction with Taq polymerase, 30 pmol pUC forward and reverse primers and o.2 mM of dG-, dC-, dTTP and 0.2mM dATP + ^{32}P-dATP. The generated radiolabeled probe was liberated from pUC19 sequences by EcoR1- Hind3 digestion followed by gel purification of the resulting 293 bp fragment.

The probe was hybridized to a membrane gridded cosmid library of genomic DNA from *A. oryzae* strain A1560 (the father of JaL142) (WO96/29391). A positive clone was identified on the filter when analyzed in a phosphoimager, and the clone was identified as λ31A2.

The λ31A2 cosmid DNA was propagated and used for southern analysis, using the same radiolabeled primer as above. An approximately 9 Kb Pst fragment, split by BstX (previously found in the cloned PCR fragment) into 5.8 and 3.2 kb fragments both lightening up with the probe, was identified and cloned into Pst cut pUC19, giving a plasmid named pUC19msh2P. The insert was sequentially sequenced, starting with primers pointing out from the previously determined sequence, followed by primers based on the sequences determined in the last run:

130740 (SEQ ID NO 12): GCTCGAAACATCCAACATCC
130741 (SEQ ID NO 13): GCTGTGAATCACTTGCACC
131928 (SEQ ID NO 14): CTTCATAAACTGCGACAAATCATGC
131929 (SEQ ID NO 15): GGAGGAGCATCTTCGC
131930 (SEQ ID NO 16): GGAACTTGAAGACTTTACTTCATCC
134608 (SEQ ID NO 17): CCAGAAACTCGCTAACACC
134609 (SEQ ID NO 18): GTGCTTTGCGGACGC
134610 (SEQ ID NO 19): CAGGACAGTAGGACGC
135320 (SEQ ID NO 20): CGAGCGATGAACTCTGC
135321 (SEQ ID NO 21): GCGTTGGTGGATTATCC
136105 (SEQ ID NO 22): CGTTGCATCTATCATATACC
136106 (SEQ ID NO 23): GGTATATGATAGATGCAACGC

The 3825 bp sequence hereby determined (SEQ ID NO 1) was translated in the frame previously determined in the PCR

fragment. The resulting protein (SEQ ID NO 2) called Ao.MSH2 was aligned to the protein sequences of known mismatch repair proteins in Figure 3. From the alignment in shown in Figure 3 the cloned and sequenced DNA clearly encompasses the coding sequence for a homolog of yeast, man and mouse mismatch repair proteins, with one intron in the N-terminal part. The position of the intron was deduced by the standard splice rules, and constitutes the only possibility.

EXAMPLE 3:

Disruption of the gene cloned in example 1 on the chromosome of an Aspergillus oryzae cell:

For the disruption experiment the msh2 CDS was deleted from pUC19msh2P (see example 2) by PCR, introducing a Not1 site instead. This was done by the primers:

137208 (SEQ ID NO 24): 5' P-CCGCGTCTCCAACAAGATGAATGG
137207 (SEQ ID NO 25): 5' P-CCGCTTTCTCGGGGTCATAGC

In a Pwo polymerase based PCR reaction with 2,5 mM $MgSO_4$ and 50 pg pUC19msh2P (conditions according to the manufacturer):

PCR-cycle profile: [96°C; 2min - 4 cycles of (94°C; 30s - 52°C;30s - 72°C; 3min) - 25 cycles of (94°C; 30s - 59°C; 30s - 72°C; 3min) - 72°C; 10min]

The resulting PCR product of appr. 8.9 Kb was isolated, ligated into pUC19, and transformed into *E. coli* XL1. From the resulting transformants pMsh2Δ was isolated, and the correctness of the new junction and its surroundings verified by sequencing [primer 138149 (SEQ ID NO 26): CCTTTCCACTTTAATCCTAAGC]. (Xl1/pMsh2Δ: Lac3073).

In this construct it the *A.oryzae* pyrG (WO 96/29391) is inserted into the NotI site.

By using this construct the chromosomal gene is deleted in an Aspergillus oryzae cell according to standard techniques

known in the art for crossing in such a deleted gene on the chromosome by homologous recombination (Miller, B.L., et al., 1985 Mol. and Cell. Biol. 5:1714-1721).

EXAMPLE 4:

Construction of a plasmid comprising the mismatch repair gene shown in SEQ ID N0 1, the AMA1 replication initiating sequence; and the AmdS selectable marker:

The plasmid constructed as described below is highly suitable for making a filamentous fungal cell wherein the mismatch repair system may be transitorily inactivated, wherein this plasmid may be inserted into a mismatch disrupted strain of example 3 when the mismatch repair system shall be activated and deleted from the strain when the mismatch repair system shall be inactivated.

Disruption of the mismatch repair gene may cause the accumulation of new chromosomal mutations, thus such a strain might be genetically unstable. Consequently it was decided to perform the chromosomal disruption in a strain where mismatch repair gene was expressed from an extra chromosomal element readily lost when the Δmismatch repair phenotype was wanted.

The extra-chromosomal element was here a plasmid comprising AMA1 as replication initiating sequence and AmdS as selectable marker.

For this purpose the mismatch repair gene (SEQ ID NO 1) was cloned into an autonomously replicating construct harbouring one AMA1 repeat.

From pMT1505 (See example 5 below for description of pMT1505) the following fragments were isolated and ligated together:

5.16 kb NotI-[Hind3]* + 3.515 kb [Sal]*-BamH1 + 757bp BamH1- NotI

[ ]* indicates that the site has been filled in by Klenow-polymerase and dNTP

From this ligation reaction pMT1505DHS was isolated (LaC 3212), and the mismatch repair expression cassette was introduced as a BamH1 - Mun1 fragment in the corresponding

sites in pMT1505DHS, resulting in the plasmid pAma-msh2 (LaC 3216).

Aspergillus oryzae JaL250 (see example 5) was transformed AmdS+ with pAma-msh2, and the transformants cheked for the ability to lose the amdS character when unselected (50% of the transformants), indicating the maintanance of this plasmid as extra chromosomal. (LaC3244 keep on acetamide + uridine).

EXAMPLE 5:

Construction of plasmid pMT1505 used in example 4:

Plasmids

pMT1505: constructed as described below in Example 5

pHelp1: contains the *pyrG* gene from *A. oryzae* as a selective marker and the AMA1 sequences which enable autonomous replication in *A. nidulans* as described by Gems, D., *et al.* (1991. Gene 98: 61-67)

pToC68: as described in EP 0 531 372 (Novo Nordisk A/S)

Strains

JaL250: a derivative of *Aspergillus oryzae* A1560 in which the *pyrG* gene has been inactivated, as described in WO 98/01470

DH5a: an *E. coli* host cell purchased from GIBCO BRL (Life Technologies, Inc., Rockville MD)

pMT1466 was constructed by inserting an SphI/NarI fragment from pHelp1 into pToC68. pMT1489 was constructed by digesting pMT1466 with SphI and StuI, then religating. pMT1500 was constructed by digesting pMT1489 with AatII and NarI and ligating a linker. pMT1504 was constructed by digesting pMT1500 with NheI and religating.

pMT1505 was constructed by inserting a 2.7 kb XbaI fragment containing the *amdS* encoding gene from *A. nidulans* genomic DNA (Corrick, C.M., et al. 1987, Gene 53:63-71) into pMT1504 which had been cut with NheI.

EXAMPLE 6:

Deletion of part of the *msh*II gene on the chromosome

The plasmid p418MsHII (from lac3159) is cut with SalI and XhoI and treated with calf-intestinal phosphatase. In this manner part of the *msH*II gene is cut out. The large band (6400 bp) containing the vector and most of the *msH*II gene is isolated from a 1% agarose gel.

The plasmid pJal554 was constructed by ligating a SpeI/SspBI cut fragment (5330 bp) from pSO2 with a Asp718/NheI cut fragment (316 bp) from pSO2. Plasmid pJal554 is cut with SalI and a 2350 bp band which contains the pyrG gene is isolated on a 1% agarose gel. The 2350bp band with pyrG is ligated with the cut p418MsHII plasmid and transformed into *E. coli*. The right *E. coli* transformant is identified by restriction analysis and a plasmid preparation is made from this transformant.

The plasmid thus prepared is cut with *Eco*RI in order to linearize the plasmid before it is transformed into for example *Aspergillus oryzae* Jal250. Transformants are selected on minimal plates.

A transformant where a double crossover event has taken place is identified by making an *Aspergillus* chromosomal DNA prep followed by a PCR screen for full-length *msh*II gene using appropriate primers. A Southern blot is made using chromosomal DNA which is randomly fragmented with appropriate enzymes as well as appropriate probes for the deleted *msH*II fragment (which is not there any longer) as well as a positive control probe.

In order to determine an increased mutation frequency in the strain with an inactivated *msH*II gene, a screen for mutations in the niaD gene is made. This is done by growing the parent strain *Aspergillus* Jal250 and the *msH*II inactivated strain on plates.

A spore-suspension is made and aliquotes of spores are plated on chlorate-containing plate as described by Unkles et al. (S. E.Unkles, E.C.Campbell. Y.M.J.T. de Ruite Jacobs, M.

Broekhuisen, J.A. Macro, D.Carrez, R. Contreras, C.A.M.J.J. van den Hondel J.R. Kinghorn. The development of a homologous transformation system for *Aspergillus oryzae* based on nitrate assimilation pathway: A convenient and general selection system for filamentous fungal transformation. Molecular and general genetics V:218 p 99-104 (1989)).

The strain with no expression of the MsHII protein will have a higher rate of niaD mutations (more chlorate resistant clones), than the control strain.

EXAMPLE 7:

Making *In Vivo* antisense *msH*II RNA to inhibit translation of *msH*II mRNA using the TAKA promoter to drive transcription of the Anti-sense mRNA

Anti-sense RNA expression is a well known way to down regulate expression of any gene *in vivo* (The design of Antisense RNA , Georg Sczakiel , Antisense and nucleic acid drug development V. 7 P. 439-444 (1997))

A pcr fragment is made using the oligo´s:
000120j2 (SEQ ID NO 27): TCTGCGAATCGCTTGGATCCCGAACGCGACAACAC,
000120j4 (SEQ ID NO 28): GAGCTCAGATCTCTTAGGTTCTGGACGAGAAGA,
and pUC19msh2P as template. This PCR fragment contains the 5 `end of the *msH*II gene including the presumed part of 5´ *msH*II mRNA. Another PCR fragment is made using the oligo´s:
000120j3 (SEQ ID NO 29): GTTGTCGCGTTCGGGATCCAAGCGATTCGCAGAAG,
1298-TAKA (SEQ ID NO 30): GCAAGCGCGCGCAATACATGGTGTTTTGATCAT,
and pENI1298 as template (PCT DK99/00552).
Both PCR reactions are done using PWO polymerase according to the manufacturers manual (Boehringer-Mannheim).

The PCR fragments are purified using the Qiagen PCR purification kit (Qiagen). The two PCR fragments are mixed and a third PCR reaction is done with primer 1298-TAKA and 000120j4. In this manner the two PCR fragments are assembled.

The assembled PCR fragment is cut with BssHII and BglII, and purified from a 1.5 % agarose gel and ligated with pENI1298 which was cut with BssHII and Bgl II (purified from 1 % agarose

gel). The ligation mixture is transformed into *E. coli*. A DNA-prep is made of each of the resulting *E. coli* transformants. The assembled PCR fragment is sequenced to confirm that no unwanted mutations are introduced during the procedure. The correct construct contains the TAKA promoter, which drives the transcription of the *msH*II anti-sense mRNA.

The resulting plasmid is transformed into for example *Aspergillus oryzae* Ja1250 along with pENI1298 as control, and transformant are selected on minimal plates. The resulting transformants are isolated on minimal plates and incubated at 37°C until they sporulate.

In order to determine an increased mutation frequency in the strain, where the translation of the *msH*II mRNA is impeded due to *msH*II anti-sense RNA expression, a screen for mutations in the niaD gene is made. A spore-suspension is prepared of the control transformants (pENi1298) and of the *msH*II Anti-sense RNA transformants, and equal amounts of spores are plated on to chlorate-containing plate as described by Unkles et al. (S. E.Unkles, E.C.Campbell. Y.M.J.T. de Ruite Jacobs, M. Broekhuisen, J.A. Macro, D.Carrez, R. Contreras, C.A.M.J.J. van den Hondel J.R. Kinghorn. The development of a homologous transformation system for *Aspergillus oryzae* based on nitrate assimilation pathway: A convenient and general selection system for filamentous fungal transformation. Molecular and general genetics V:218 p 99-104 (1989)).

The strain with no or low expression of the MsHII protein will have a higher rate of niaD mutations (more chlorate resistant clones), than the control strain.

CLAIMS

1. A filamentous fungal cell, wherein a gene involved in the mismatch repair system has been inactivated and in which the gene involved in the mismatch repair system comprises:
   (a) a DNA sequence encoding the polypeptide sequence shown in positions 683-758 of SEQ ID NO 2; or
   (b) a DNA sequence encoding a polypeptide sequence which is at least 70% identical to the polypeptide sequence shown in positions 683-758 of SEQ ID NO 2.

2. A filamentous fungal cell, wherein a gene involved in the mismatch repair system has been inactivated and in which the gene involved in the mismatch repair system comprises:
   (a) a DNA sequence encoding the polypeptide sequence shown in positions 1-940 of SEQ ID NO 2; or
   (b) a DNA sequence encoding a polypeptide sequence which is at least 70% identical to the polypeptide sequence shown in positions 1-940 of SEQ ID NO 2.

3. The filamentous fungal cell of claim 1 or 2, wherein the gene involved in the mismatch repair is defective.

4. The filamentous fungal cell of claim 1 or 2, wherein the gene involved in the mismatch repair has been inactivated transitorily.

5. The filamentous fungal cell of any of the preceding claims, wherein the filamentous fungal cell is a strain of *Fusarium* or more preferably *Aspergillus*.

6. A process for preparing a filamentous fungal cell population wherein individual cells in the population comprise individually different DNA sequences of interest representing a DNA library of interest comprising following steps:
   (a) placing individually different DNA sequences of interest in a filamentous fungal cell population comprising a filamentous fungal cell of claim 1 or 2; and

(b) growing the population of (a) for a period of time allowing an individual DNA sequence of interest in the population to be duplicated at least once under conditions wherein the mismatch repair system gene of claim 1 or 2 has been inactivated.

7. The process of claim 6, wherein the mismatch repair system under step (b) is defective.

8. The process of claim 6, wherein the mismatch repair system under step (b) has been inactivated transitorily.

9. The process of any of claims 6-8, wherein the filamentous fungal cell is a strain of *Fusarium* or more preferably *Aspergillus*.

10. The process of any of claims 6-9, wherein, under step (b) of claim 6, there is an *in vivo* intergenic recombination of partially homologous DNA sequences of interest.

11. A process for production of a polypeptide of interest comprising the steps of claim 6 and wherein the DNA sequences of interest encode a polypeptide of interest and which further comprises following step:

(c) selecting from the resultant population of filamentous fungal cells of step (b) of claim 6 a desired polypeptide of interest.

12. The process of claim 11, which further comprises following steps:

(d) an optionally step comprising modifying the amino acid sequence of the desired selected polypeptide of interest according to a particularly further specific need;

(e) placing the DNA sequence encoding the polypeptide of interest of step (c) of claim 11 or the modified polypeptide of interest of step (d) into a filamentous

fungal cell which is suitable for large scale production of the polypeptide of interest;

(f) cultivating the filamentous fungal cell of step (e) in a fermentor of at least 10000 m3 under conditions permitting expression of the polypeptide of interest; and

(g) isolating the polypeptide of interest.

13. The process of claim 12, wherein the filamentous fungal cell which is suitable for large scale production of the polypeptide of interest of step (e) of claim 12 is another filamentous fungal cell as compared to the filamentous fungal cell of step (a) of claim 6.

14. The process of any of claims 11-13, wherein the polypeptide of interest is a polypeptide derived from a filamentous fungal cell.

15. The process of any of claims 11-14, wherein the polypeptide of interest is an enzyme, such as an amylase, a protease, a cellulase, a lipase, a xylanase; a phospholipase.

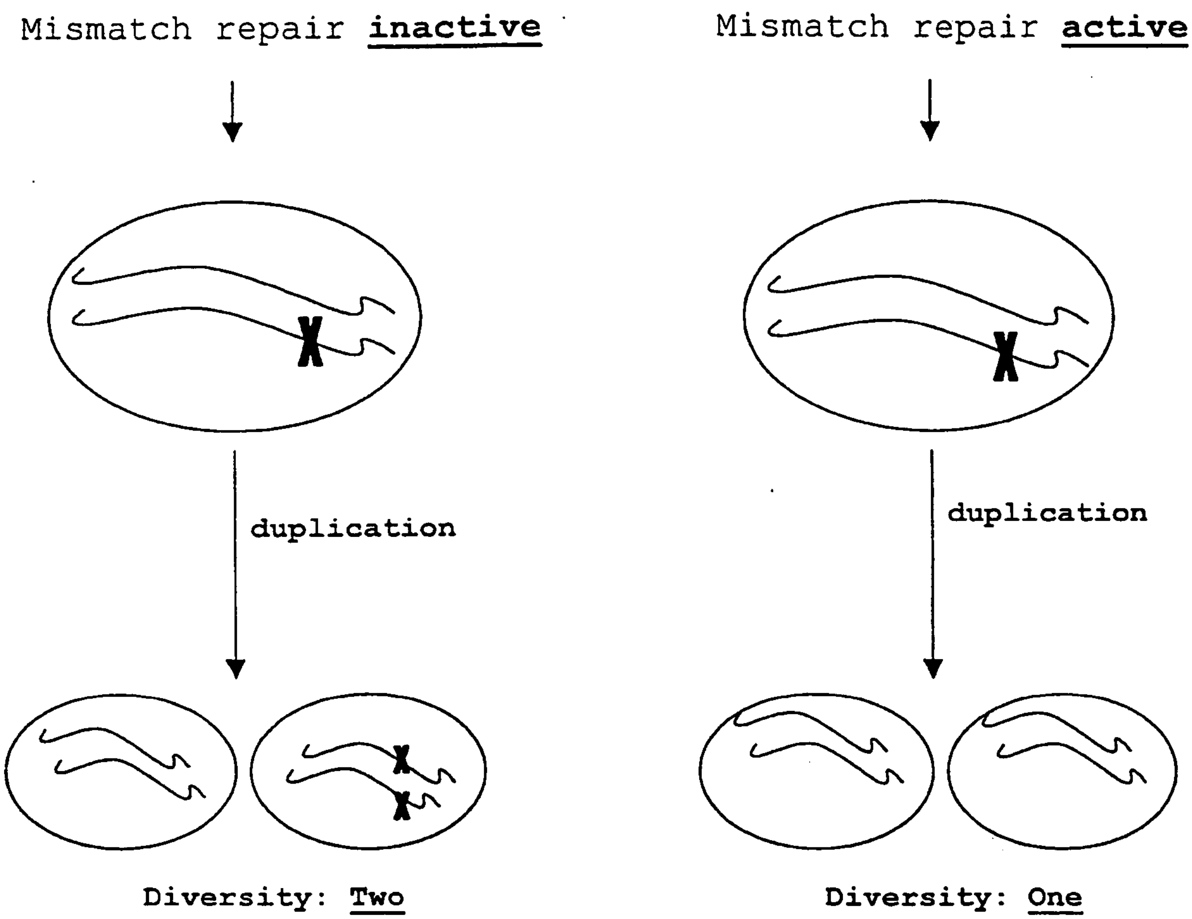
Mismatch repair **inactive**
Mismatch repair **active**
duplication
duplication
Diversity: Two
Diversity: One

Fig. 1

2/8

```
                                                                                    70
                                                                                     |
Contig# 1         •••••••••  ••||• • •• ••••|•••••••••••• •|•••||••||•| |••••|••••••••••••••••
'msh2'Ao-col10    AQIGCFVPCTEAELTIFDCILARVGASDSQLKGVSTFMAEMLETSNILKSATSESLIIIDELGRGTSTYDGFGLAW
'msh2'Ao-col13    AQIGCFVPCTEAELTIFDCILARVGASDSQLKGVSTFMAEMLETSNILKSATSESLIIIDELGRGTSTYDGFGLAW
'msh2'Ao-col15    AQIGCFVPCTEAELTIFDCILARVGASDSQLKGVSTFMAEMLETSNILKSATSESLIIIDELGRGTSTYDGFGLAW
msh2-human.p      AQIGCFVPCESAEVSIVDCILARVGAGDSQLKGVSTFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAW
S.c. msh2         AQIGCFVPCEEAEIAIVDAILCRVGAGDSQLKGVSTFMVEILETASILKNASKNSLIIVDELGRGTSTYDGFGLAW
```

Fig. 2

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                         10        20        30        40        50        60
                          |         |         |         |         |         |
Contig# 1      o|     o     |   o  o | |  ||| ||  o|o| o|oo o|  o  o | o  o| o ||||
S.c. msh2      MSSTRPELKFSDVSEERNFYKKYTGLPKKPLK-TIRLVDKGDYYTVIGSDAIFVADSVYHTQSVLK
Ao.MSH2        MSS-RPELKV-D--DEVGFIRFYRSLAANSNDETIRVFDRGDWYSAHGAKAEFIARTVYKTTSILR
msh2 mus.p     MAVQPKETLQLEGAAEAGFVRFFEGMPEKPST-TVRLFDRGDFYTAHGEDALLAAREVFKTQGVIK
msh2-human.p   MAVQPKETLQLESAAEVGFVRFFQGMPEKPTT-TVRLFDRGDFYTAHGEDALLAAREVFKTQGVIK

                  70        80        90       100       110       120       130
                   |         |         |         |         |         |         |
Contig# 1          ||   |  |     o |o   | ||||  o|   ||o|                |o o  ooo
S.c. msh2      NCQLDPVTAKNFHEPTKYVTVSLQVLATLLKLCLLDLGYKVEIY----------DKGWKLIKSASP
Ao.MSH2        N--LGRSDSGGLPS----VTMSVTVFRNFLREALFRLNKRIEIW----GSVGTGKGHWKLVKQASP
msh2 mus.p     Y--MGPAGSKTLQS----VVLSKMNFESFVKDLLLVRQYRVEVYKNKAGNKASKENEWYLAFKASP
msh2-human.p   Y--MGPAGAKNLQS----VVLSKMNFESFVKDLLLVRQYRVEVYKNRAGNKASKENDWYLAYKASP

                     140       150       160       170       180       190
                       |         |         |         |         |         |
Contig# 1      oo| | ||  |        | ||  | ||o|     |  |oo | o|    |o| || |o| |oo|
S.c. msh2      GNIEQVNELM----NMNIDSSIIIASLKVQWNSQDGNCIIGVAFIDTTAYKVGMLDIVDNEVYSNL
Ao.MSH2        GNLQDVEEELGSVGGLSMDSAPIILAVKIS-AKAAEARSVGVCFADASVRELGVSEFLDNDIYSNF
msh2 mus.p     GNLSQFEDIL--FGNNDMSASVGVMGIKMA-VVDGQ-RHVGVGYVDSTQRKLGLCEFPENDQFSNL
msh2-human.p   GNLSQFEDIL--FGNNDMSASIGVVGVKMS-AVDGQ-RQVGVGYVDSIQRKLGLCEFPDNDQFSNL
```

Fig. 3

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              200       210       220       230       240       250       260
               |         |         |         |         |         |         |
Contig# 1     o|||oo|o ooo|            || o|  | |  o  ||    |||| oo|  oo |oo
S.c. msh2     ESFLIQLGVKECLVQDLTSNSNSNAEMQKVINVIDRCGCVVTLLKNSEFSEKDVELDLTKLL----
Ao.MSH2       ESLIIQLGVKECLVQ--MDANKKDVELGKIRAIADSCGIAISERPVADYGVKDIEQDLTRLLRDER
msh2 mus.p    EALLIQIGPKECV----LPGGETTGDMGKLRQVIQRGGILITERKRADFSTKDIYQDLNRLLKGKK
msh2-human.p  EALLIQIGPKECV----LPGGETAGDMGKLRQIIQRGGILITERKKADFSTKDIYQDLNRLLKGKK

                  270       280       290       300       310       320       330
                   |         |         |         |         |         |         |
Contig# 1     |    |  oo|   |||||| |o|o |o ||||  | o||o     | ||oooo |o||oooo
S.c. msh2     GDDL-ALSLPQKYSKLSMGACNALIGYLQLLSEQDQVGKYELVEHKLKEFMKLDASAIKALNLFPQ
Ao.MSH2       S----AGTLPQTELKLAMGSASALIKYLGVMTDPTNFGQYQLYQHDLSQFMKLDSSALRALNLMPG
msh2 mus.p    GEQINSAALPEMENQVAVSSLSAVIKFLELLSDDSNFGQFELATFDFSQYMKLDMAAVRALNLFQG
msh2-human.p  GEQMNSAVLPEMENQVAVSSLSAVIKFLELLSDDSNFGQFELTTFDFSQYMKLDIAAVRALNLFQG

                      340       350       360       370       380       390
                       |         |         |         |         |         |
Contig# 1     | ||   o                  oo  ooo ooo  o oo| |o|oooo |   o| o |oo|
S.c. msh2     GPQNPFGSNNLAVSGFTSAGNSGKVTSLFQLLNHCKTNAGVRLLNEWLKQPLTNIDEINKRHDLVD
Ao.MSH2       PRDGSK-SM-----------------SLFGLLNHCKTPVGSRLLAQWLKQPLMDLAEIEKRQQLVE
msh2 mus.p    SVEDTTGSQ-----------------SLAALLNKCKTAQGQRLVNQWIKQPLMDRNRIEERLNLVE
msh2-human.p  SVEDTTGSQ-----------------SLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVE
```

Fig. 3 (Continued)

```
                400       410       420       430       440       450       460
                  |         |         |         |         |         |         |
Contig# 1      ||| oooo |  | o  |oo| oo|o|||||  oo|o  ||oo   ||o  || |    |
S.c. msh2      YLIDQIELRQMLTSEYLPMIPDIRRLTKKLNKRG-NLEDVLKIYQFSKRIPEIVQVFTSFLEDDSP
Ao.MSH2        AFVVNTELRQTMQEEHLRSIPDLYRLAKRFQRKQANLEDVVRVYQVAIRLPGFVNSLENVMDEEYQ
msh2 mus.p     AFVEDSELRQSLQEDLLRRFPDLNRLAKKFQRQAANLQDCYRLYQGINQLPSVIQALEKY-EGRHQ
msh2-human.p   AFVEDAELRQTLQEDLLRRFPDLNRLAKKFQRQAANLQDCYRLYQGINQLPNVIQALEKH-EGKHQ

                   470       480       490       500       510       520
                     |         |         |         |         |         |
Contig# 1            | |   | o     ||o||oo|ooo|o|o  o o|oo||o  o| |o  |o  || o
S.c. msh2      TEPVNELVRSVWLAPLSHHVEPLSKFEEMVETTVDLDAYEENNEFMIKVEFNEELGKIRSKLDTLR
Ao.MSH2        T-----PLETEYTSNLRSHSDSLAKLEEMVETTVDLDALE-NHEFIIKPEFDESLRIIRKKLDKLR
msh2 mus.p     A-----LLLAVFVTPLIDLRSDFSKFQEMIETTLDMDQVE-NHEFLVKPSFDPNLSELREVMDGLE
msh2-human.p   K-----LLLAVFVTPLTDLRSDFSKFQEMIETTLDMDQVE-NHEFLVKPSFDPNLSELREIMNDLE

               530       540       550       560       570       580       590
                 |         |         |         |         |         |         |
Contig# 1         |      o |o| o  o|| o||     o    o|o ||   |o|||||     o o| o|
S.c. msh2      DEIHSIHLDSAEDLGFDPDKKLKLENHHLHGWCMRLTRNDAKELRKHKKYIELSTVKAGIFFSTKQ
Ao.MSH2        HDMGVEHRRVARDLDQDIEKKLFLENHRVHGWCFRLTRNESGCIRNKREYQECSTQKNGVYFTTST
msh2 mus.p     KKMQSTLINAARGLGLDPGKQIKLDSSAQFGYYFRVTCKEEKVLRNNKNFSTVDIQKNGVKFTNSE
msh2-human.p   KKMQSTLISAARDLGLDPGKQIKLDSSAQFGYYFRVTCKEEKVLRNNKNFSTVDIQKNGVKFTNSK
```

Fig. 3 (Continued)

```
                  600       610       620       630       640       650       660
                    |         |         |         |         |         |         |
Contig# 1     | ||  o       |o|  o ||o|o||o||  o      | o  o|o|oo | oooo o  oo oo o
S.c. msh2     LKSIANETNILQKEYDKQQSALVREIINITLTYTPVFEKLSLVLAHLDVIASFAHTSSYAPIPYIR

Ao.MSH2       MQTLRREHDQLSSNYNRTQTGLVNEVVNVAASYCPVLERLAGVIAHLDVIVSFAHASVHAPTPYAR

msh2 mus.p    LSSLNEEYTKNKGEYEEAQDAIVKEIVNISSGYVEPMQTLNDVLAHLDAIVSFAHVSNAAPVPYVR

msh2-human.p  LTSLNEEYTKNKTEYEEAQDAIVKEIVNISSGYVEPMQTLNDVLAQLDAVVSFAHVSNGAPVPYVR


                       670       680       690       700       710       720
                         |         |         |         |         |         |
Contig# 1     o |   |       o   |oo|  |o|oo|||oo ooo |   |    o oooooooooooooooo ooo
S.c. msh2     PKLHPMDSERRTHLISSRHPVLEMQDDISFISNDVTLESGKGDFLIITGPNMGGKSTYIRQVGVIS

Ao.MSH2       PKMHPRGTGNTV-LKEARHPCMEMQDDISFITNDVALVRDESSFLIITGPNMGGKSTYIRQIGVIA

msh2 mus.p    PVILEKGKGRII-LKASRHACVEVQDEVAFIPNDVHFEKDKQMFHIITGPNMGGKSTYIRQTGVIV

msh2-human.p  PAILEKGQGRII-LKASRHACVEVQDEIAFIPNDVYFEKDKQMFHIITGPNMGGKSTYIRQTGVIV


                730       740       750       760       770       780       790
                  |         |         |         |         |         |         |
Contig# 1     oooo oooooo   oo||o o oo oooo|oooooooooo o|ooo||oo||o| |oooo|ooooo
S.c. msh2     LMAQIGCFVPCEEAEIAIVDAILCRVGAGDSQLKGVSTFMVEILETASILKNASKNSLIIVDELGR

Ao.MSH2       LMAQTGCFVPCTEAELTIFDCILARVGASDSQLKGVSTFMAEMLETSNILKSATSESLIIIDELGR

msh2 mus.p    LMAQIGCFVPCESAEVSIVDCILARVGAGDSQLKGVSTFMAEMLETSSILRSATKDSLIIIDELGR

msh2-human.p  LMAQIGCFVPCESAEVSIVDCILARVGAGDSQLKGVSTFMAEMLETASILRSATKDSLIIIDELGR
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Fig. 3 (Continued)

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                       800       810       820       830       840       850
                         |         |         |         |         |         |
Contig# 1     oooooooooooooo|| o | o  o |ooooooooo o||| o  o|o|oo o
S.c. msh2     GTSTYDGFGLAWAIAEHIASKIGCFALFATHFHELTELSEKLPN-VKNMHVVAHIEKNLKEQKHDD

Ao.MSH2       GTSTYDGFGLAWAISEHIVTEIRCFGLFATHFHELTALADRYPKSVKNLHVVAFIGDGTDDDSEDK

msh2 mus.p    GTSTYDGFGLAWAISDYIATKIGAFCMFATHFHELTALANQIP-TVNNLHVTALTTEET-------

msh2-human.p  GTSTYDGFGLAWAISEYIATKIGAFCMFATHFHELTALANQIP-TVNNLHVTALTTEET-------

              860       870       880       890       900       910       920
               |         |         |         |         |         |         |
Contig# 1           |o|oo|o  o| oooooooooo|  oo   ||  o||oo oo|||
S.c. msh2     ----EDITLLYKVEPGISDQSFGIHVAEVVQFPEKIVKMAKRKANELDDLKTNNEDLK------KA

Ao.MSH2       KSKRNQVTLLYRVEPGICDQSFGIHVAELVRFPEKVVNMARQKAEELEDFTS-SEQQDQQSSMAID

msh2 mus.p    ------LTMLYQVKKGVCDQSFGIHVAELANFPRHVIACAKQKALELEEFQNIGTSLGCDEAEPAA

msh2-human.p  ------LTMLYQVKKGVCDQSFGIHVAELANFPKHVIECAKQKALELEEFQYIGESQGYDIMEPAA
```

Fig. 3 (Continued)

```
                   930       940       950       960       970       980       990
                    |         |         |         |         |         |         |
Contig# 1      o    o ||o      ||   | o o       |   o     o | |   |    |   |  |
S.c. msh2      KLSLQEVNEGNIRLKALLKEWIRKVKEEGLHDPSKITEEASQHKIQELLRAIANEPEKENDNYLEI
Ao.MSH2        KYSQEEVEEGS----ALLKAMLLKWKSETE---SSGKELTVEEKRQIMRDLVKADEKLQANKVFQG
msh2 mus.p     KRRCLEREQGE----KIILEFLSKVKQVPF---TAMSEESISAKLKQLKAEVVAKNNSFVNEIISR
msh2-human.p   KKCYLEREQGE----KIIQEFLSKVKQMPF---TEMSEENITIKLKQLKAEVIAKNNSFVNEIISR

Contig# 1       o
S.c. msh2      YKSPCCYN
Ao.MSH2        IK--A--L
msh2 mus.p     IKAPA--P
msh2-human.p   IKV-T--T
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Fig. 3 (Continued)

SEQUENCE LISTING

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tcaagacttc tactgtttaa tgtgtgtttt ggatgtttgg tgttgctggg ttggataccc 360

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agtacttcag ttcctatgtg taggcgtttt ttggacctat cactttgtag agttctaacg 540

ggtgctatta ttggccaatg atacttcaat atatcgcgaa cgcgacaaca cgtgaccgcg 600

ttgccacgga gcgcgtctcc tggaattatc gaaatagatc gatcacggca gagctaatgg 660

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                                          Met Ser Ser Arg Pro
                                           1               5
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gaa ctt aag gtaagtaaac aagacaaccg gtctctcgaa cattcaataa          763
Glu Leu Lys

caattaaccc tgtttag gtt gac gac gaa gtc ggc ttc att cgt ttt tac   813
                   Val Asp Asp Glu Val Gly Phe Ile Arg Phe Tyr
                            10                  15

cgt tcc ctc gca gca aat agc aac gat gaa act att cgc gtt ttc gac   861
Arg Ser Leu Ala Ala Asn Ser Asn Asp Glu Thr Ile Arg Val Phe Asp
 20                  25                  30                  35

cgc ggt gac tgg tac tct gcc cat ggc gcc aaa gca gag ttc atc gct   909
Arg Gly Asp Trp Tyr Ser Ala His Gly Ala Lys Ala Glu Phe Ile Ala
                40                  45                  50

cgc act gtg tac aag acc acc tct ata ctc cgc aat cta ggt cgc agc   957
Arg Thr Val Tyr Lys Thr Thr Ser Ile Leu Arg Asn Leu Gly Arg Ser
            55                  60                  65

gac tca gga ggc ctt ccc tcc gtc acc atg agt gtc acc gtc ttc cgt   1005
Asp Ser Gly Gly Leu Pro Ser Val Thr Met Ser Val Thr Val Phe Arg
        70                  75                  80

aac ttt ctc cgc gaa gct ctc ttc cga ctc aac aag cgc att gaa atc   1053
Asn Phe Leu Arg Glu Ala Leu Phe Arg Leu Asn Lys Arg Ile Glu Ile
    85                  90                  95

tgg ggc tca gtc gga acg ggc aag ggt cat tgg aag ctg gta aag caa   1101
Trp Gly Ser Val Gly Thr Gly Lys Gly His Trp Lys Leu Val Lys Gln
100                 105                 110                 115

gct agc ccg gga aac ctc caa gat gtg gaa gaa gag ttg ggc agc gtt   1149
Ala Ser Pro Gly Asn Leu Gln Asp Val Glu Glu Glu Leu Gly Ser Val
                120                 125                 130

ggt gga tta tcc atg gac tcg gct cca att atc cta gca gtg aag atc   1197
Gly Gly Leu Ser Met Asp Ser Ala Pro Ile Ile Leu Ala Val Lys Ile
            135                 140                 145

tcg gcc aag gcc gca gag gct agg agt gtg gga gtg tgc ttt gcg gac   1245
Ser Ala Lys Ala Ala Glu Ala Arg Ser Val Gly Val Cys Phe Ala Asp
        150                 155                 160

gca agt gta cgg gaa ctc ggt gtt agc gag ttt ctg gat aac gat atc   1293
Ala Ser Val Arg Glu Leu Gly Val Ser Glu Phe Leu Asp Asn Asp Ile
    165                 170                 175

tat tcc aac ttt gag tcg ctt att atc caa ctc ggg gtg aag gag tgt   1341
Tyr Ser Asn Phe Glu Ser Leu Ile Ile Gln Leu Gly Val Lys Glu Cys
180                 185                 190                 195

ttg gtg cag atg gat gct aat aag aag gat gtt gag ctg gga aag att   1389
Leu Val Gln Met Asp Ala Asn Lys Lys Asp Val Glu Leu Gly Lys Ile
                200                 205                 210

cgg gct att gcg gat agt tgt ggg atc gct atc tcc gag agg ccg gtg   1437
Arg Ala Ile Ala Asp Ser Cys Gly Ile Ala Ile Ser Glu Arg Pro Val
            215                 220                 225
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gct gat tat ggt gtc aag gat att gag cag gat ctg acg agg ttg ttg    1485
Ala Asp Tyr Gly Val Lys Asp Ile Glu Gln Asp Leu Thr Arg Leu Leu
        230                 235                 240

agg gat gaa cgg tcg gct ggt acg ctg ccg cag acg gag cta aag ctt    1533
Arg Asp Glu Arg Ser Ala Gly Thr Leu Pro Gln Thr Glu Leu Lys Leu
    245                 250                 255

gcg atg ggc tcg gcg tct gcg ttg atc aag tac ctt ggg gtt atg acg    1581
Ala Met Gly Ser Ala Ser Ala Leu Ile Lys Tyr Leu Gly Val Met Thr
260                 265                 270                 275

gat cct aca aac ttc ggc cag tac cag ctc tat cag cat gat ttg tcg    1629
Asp Pro Thr Asn Phe Gly Gln Tyr Gln Leu Tyr Gln His Asp Leu Ser
                280                 285                 290

cag ttt atg aag ttg gat tcg tcg gcg ctg cgt gct ctt aac ctt atg    1677
Gln Phe Met Lys Leu Asp Ser Ser Ala Leu Arg Ala Leu Asn Leu Met
            295                 300                 305

cct ggt ccg cgg gac gga tcg aag tct atg agt ttg ttt ggt ttg ttg    1725
Pro Gly Pro Arg Asp Gly Ser Lys Ser Met Ser Leu Phe Gly Leu Leu
        310                 315                 320

aat cac tgc aag acc cct gtt ggt agc cgg ttg ctt gcg cag tgg ctg    1773
Asn His Cys Lys Thr Pro Val Gly Ser Arg Leu Leu Ala Gln Trp Leu
    325                 330                 335

aaa cag ccg ttg atg gat ctg gcg gag atc gag aag aga cag cag ctt    1821
Lys Gln Pro Leu Met Asp Leu Ala Glu Ile Glu Lys Arg Gln Gln Leu
340                 345                 350                 355

gtt gag gcg ttt gtt gtt aac acg gag ctc aga cag act atg cag gag    1869
Val Glu Ala Phe Val Val Asn Thr Glu Leu Arg Gln Thr Met Gln Glu
                360                 365                 370

gag cat ctt cgc tcc ata ccg gat ctg tat aga cta gcg aag cgg ttc    1917
Glu His Leu Arg Ser Ile Pro Asp Leu Tyr Arg Leu Ala Lys Arg Phe
            375                 380                 385

cag cgc aaa cag gca aac ttg gaa gac gtt gtg cgg gtg tac cag gtt    1965
Gln Arg Lys Gln Ala Asn Leu Glu Asp Val Val Arg Val Tyr Gln Val
        390                 395                 400

gct att cgt ttg cct ggt ttt gtc aac tct ctc gag aat gtt atg gat    2013
Ala Ile Arg Leu Pro Gly Phe Val Asn Ser Leu Glu Asn Val Met Asp
    405                 410                 415

gaa gag tat cag acg ccc ctg gag acg gag tat act tcc aac ctc cgg    2061
Glu Glu Tyr Gln Thr Pro Leu Glu Thr Glu Tyr Thr Ser Asn Leu Arg
420                 425                 430                 435

agt cac tct gat agc tta gcg aaa ctg gag gag atg gtt gag act acg    2109
Ser His Ser Asp Ser Leu Ala Lys Leu Glu Glu Met Val Glu Thr Thr
                440                 445                 450

gtt gac ctt gat gcg ctg gag aac cac gag ttc atc atc aag cct gag    2157
Val Asp Leu Asp Ala Leu Glu Asn His Glu Phe Ile Ile Lys Pro Glu
            455                 460                 465
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ttt gac gag agt ctg cgg atc atc agg aag aag ctg gac aag ctc cgt    2205
Phe Asp Glu Ser Leu Arg Ile Ile Arg Lys Lys Leu Asp Lys Leu Arg
        470                 475                 480

cat gat atg ggc gtt gag cac cgc agg gta gct cgg gac ctt gac caa    2253
His Asp Met Gly Val Glu His Arg Arg Val Ala Arg Asp Leu Asp Gln
    485                 490                 495

gat att gag aag aag ttg ttc ctg gag aac cac agg gtg cac gga tgg    2301
Asp Ile Glu Lys Lys Leu Phe Leu Glu Asn His Arg Val His Gly Trp
500                 505                 510                 515

tgc ttc cga ctt act cgc aac gag tcg gga tgc atc cgc aat aag aga    2349
Cys Phe Arg Leu Thr Arg Asn Glu Ser Gly Cys Ile Arg Asn Lys Arg
                520                 525                 530

gag tac cag gaa tgt tct aca cag aag aac ggt gtc tac ttc act acg    2397
Glu Tyr Gln Glu Cys Ser Thr Gln Lys Asn Gly Val Tyr Phe Thr Thr
            535                 540                 545

tcg act atg caa acc ttg cgc cgg gag cat gat caa ctg tcc tcg aac    2445
Ser Thr Met Gln Thr Leu Arg Arg Glu His Asp Gln Leu Ser Ser Asn
        550                 555                 560

tac aat aga act cag acc ggc ctg gtg aat gag gtc gtt aac gtt gcc    2493
Tyr Asn Arg Thr Gln Thr Gly Leu Val Asn Glu Val Val Asn Val Ala
    565                 570                 575

gcg tcc tac tgt cct gtt ttg gaa cga ctt gcc ggt gtc ata gca cac    2541
Ala Ser Tyr Cys Pro Val Leu Glu Arg Leu Ala Gly Val Ile Ala His
580                 585                 590                 595

ctc gat gtc att gta agc ttc gct cat gct tct gtt cat gcg ccg acc    2589
Leu Asp Val Ile Val Ser Phe Ala His Ala Ser Val His Ala Pro Thr
                600                 605                 610

ccc tat gct cgg ccc aag atg cac cca cga ggc acc gga aac aca gtt    2637
Pro Tyr Ala Arg Pro Lys Met His Pro Arg Gly Thr Gly Asn Thr Val
            615                 620                 625

ctc aag gaa gcg cgc cac ccc tgt atg gaa atg cag gat gat att tca    2685
Leu Lys Glu Ala Arg His Pro Cys Met Glu Met Gln Asp Asp Ile Ser
        630                 635                 640

ttc att act aat gat gtg gct ttg gtc cga gac gag tcc tcc ttc ctc    2733
Phe Ile Thr Asn Asp Val Ala Leu Val Arg Asp Glu Ser Ser Phe Leu
    645                 650                 655

atc att act ggt cct aac atg gga ggt aaa tcg act tat att cgc caa    2781
Ile Ile Thr Gly Pro Asn Met Gly Gly Lys Ser Thr Tyr Ile Arg Gln
660                 665                 670                 675

att ggt gtt atc gct ctc atg gct cag acg ggc tgc ttt gtg cct tgt    2829
Ile Gly Val Ile Ala Leu Met Ala Gln Thr Gly Cys Phe Val Pro Cys
                680                 685                 690

aca gaa gca gaa ttg acc atc ttt gac tgt atc ctt gca cgt gtt ggt    2877
Thr Glu Ala Glu Leu Thr Ile Phe Asp Cys Ile Leu Ala Arg Val Gly
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Ala Ser Asp Ser Gln Leu Lys Gly Val Ser Thr Phe Met Ala Glu Met
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ctc gaa aca tcc aac atc ctc aag tcg gca acg tcc gag tct ctt atc     2973
Leu Glu Thr Ser Asn Ile Leu Lys Ser Ala Thr Ser Glu Ser Leu Ile
    725                 730                 735

atc atc gac gag ctt ggg cgc ggt aca agc acg tat gac gga ttc ggc     3021
Ile Ile Asp Glu Leu Gly Arg Gly Thr Ser Thr Tyr Asp Gly Phe Gly
740                 745                 750                 755

cta gca tgg gcc atc tct gaa cac atc gtc aca gag att cgt tgc ttc     3069
Leu Ala Trp Ala Ile Ser Glu His Ile Val Thr Glu Ile Arg Cys Phe
                760                 765                 770

ggc ctt ttc gct act cac ttc cat gaa ttg aca gct ctc gcc gat cga     3117
Gly Leu Phe Ala Thr His Phe His Glu Leu Thr Ala Leu Ala Asp Arg
            775                 780                 785

tac ccc aag tct gtc aag aac ctg cac gta gtc gcc ttc atc ggc gat     3165
Tyr Pro Lys Ser Val Lys Asn Leu His Val Val Ala Phe Ile Gly Asp
        790                 795                 800

ggt act gat gat gac agt gaa gat aag aag tcc aag cgg aac cag gtc     3213
Gly Thr Asp Asp Asp Ser Glu Asp Lys Lys Ser Lys Arg Asn Gln Val
    805                 810                 815

act ctt ctg tac cgg gtc gaa cct ggc att tgt gac cag tca ttc ggt     3261
Thr Leu Leu Tyr Arg Val Glu Pro Gly Ile Cys Asp Gln Ser Phe Gly
820                 825                 830                 835

atc cac gtt gcc gaa ttg gtc cgc ttc ccg gag aag gtg gtc aac atg     3309
Ile His Val Ala Glu Leu Val Arg Phe Pro Glu Lys Val Val Asn Met
                840                 845                 850

gcc cgc cag aag gca gag gaa ctt gaa gac ttt act tca tcc gaa cag     3357
Ala Arg Gln Lys Ala Glu Glu Leu Glu Asp Phe Thr Ser Ser Glu Gln
            855                 860                 865

caa gac cag cag tca tcc atg gcg atc gat aaa tac tcg cag gaa gaa     3405
Gln Asp Gln Gln Ser Ser Met Ala Ile Asp Lys Tyr Ser Gln Glu Glu
        870                 875                 880

gtt gag gag ggc agt gcc ctt ctc aaa gcg atg ctg ctg aaa tgg aag     3453
Val Glu Glu Gly Ser Ala Leu Leu Lys Ala Met Leu Leu Lys Trp Lys
    885                 890                 895

tcg gag acc gag tcc tct ggt aag gag ttg aca gtg gaa gag aag cga     3501
Ser Glu Thr Glu Ser Ser Gly Lys Glu Leu Thr Val Glu Glu Lys Arg
900                 905                 910                 915

cag atc atg cgt gat ctc gtc aaa gca gat gag aag ctg caa gca aac     3549
Gln Ile Met Arg Asp Leu Val Lys Ala Asp Glu Lys Leu Gln Ala Asn
                920                 925                 930

aag gtc ttc cag ggt atc aag gct tta tag attagtattt gcgtcttttt       3599
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cttctttgga catatcgctt aggattaaag tggaaaggga gaaatcccat tcaactatat 3779

cgacataagt cacgttgaga tcgcgagtct agacgctcac cggg                  3823


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Val Phe Asp Arg Gly Asp Trp Tyr Ser Ala His Gly Ala Lys Ala Glu
        35                  40                  45
Phe Ile Ala Arg Thr Val Tyr Lys Thr Thr Ser Ile Leu Arg Asn Leu
    50                  55                  60
Gly Arg Ser Asp Ser Gly Gly Leu Pro Ser Val Thr Met Ser Val Thr
65                  70                  75                  80
Val Phe Arg Asn Phe Leu Arg Glu Ala Leu Phe Arg Leu Asn Lys Arg
                85                  90                  95
Ile Glu Ile Trp Gly Ser Val Gly Thr Gly Lys Gly His Trp Lys Leu
            100                 105                 110
Val Lys Gln Ala Ser Pro Gly Asn Leu Gln Asp Val Glu Glu Glu Leu
        115                 120                 125
Gly Ser Val Gly Gly Leu Ser Met Asp Ser Ala Pro Ile Ile Leu Ala
    130                 135                 140
Val Lys Ile Ser Ala Lys Ala Ala Glu Ala Arg Ser Val Gly Val Cys
145                 150                 155                 160
Phe Ala Asp Ala Ser Val Arg Glu Leu Gly Val Ser Glu Phe Leu Asp
                165                 170                 175
Asn Asp Ile Tyr Ser Asn Phe Glu Ser Leu Ile Ile Gln Leu Gly Val
            180                 185                 190
Lys Glu Cys Leu Val Gln Met Asp Ala Asn Lys Lys Asp Val Glu Leu
        195                 200                 205
Gly Lys Ile Arg Ala Ile Ala Asp Ser Cys Gly Ile Ala Ile Ser Glu
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Arg Pro Val Ala Asp Tyr Gly Val Lys Asp Ile Glu Gln Asp Leu Thr
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Val Met Thr Asp Pro Thr Asn Phe Gly Gln Tyr Gln Leu Tyr Gln His
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Asp Leu Ser Gln Phe Met Lys Leu Asp Ser Ser Ala Leu Arg Ala Leu
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Gly Leu Leu Asn His Cys Lys Thr Pro Val Gly Ser Arg Leu Leu Ala
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Gln Gln Leu Val Glu Ala Phe Val Val Asn Thr Glu Leu Arg Gln Thr
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Met Gln Glu Glu His Leu Arg Ser Ile Pro Asp Leu Tyr Arg Leu Ala
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Tyr Gln Val Ala Ile Arg Leu Pro Gly Phe Val Asn Ser Leu Glu Asn
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Val Met Asp Glu Glu Tyr Gln Thr Pro Leu Glu Thr Glu Tyr Thr Ser
420 425 430
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Glu Thr Thr Val Asp Leu Asp Ala Leu Glu Asn His Glu Phe Ile Ile
450 455 460
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465 470 475 480
Lys Leu Arg His Asp Met Gly Val Glu His Arg Arg Val Ala Arg Asp
485 490 495
Leu Asp Gln Asp Ile Glu Lys Lys Leu Phe Leu Glu Asn His Arg Val
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His Gly Trp Cys Phe Arg Leu Thr Arg Asn Glu Ser Gly Cys Ile Arg
515 520 525
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Ser Ser Asn Tyr Asn Arg Thr Gln Thr Gly Leu Val Asn Glu Val Val
565 570 575
Asn Val Ala Ala Ser Tyr Cys Pro Val Leu Glu Arg Leu Ala Gly Val
580 585 590
Ile Ala His Leu Asp Val Ile Val Ser Phe Ala His Ala Ser Val His
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Ala Pro Thr Pro Tyr Ala Arg Pro Lys Met His Pro Arg Gly Thr Gly
610 615 620
Asn Thr Val Leu Lys Glu Ala Arg His Pro Cys Met Glu Met Gln Asp
625 630 635 640
Asp Ile Ser Phe Ile Thr Asn Asp Val Ala Leu Val Arg Asp Glu Ser
645 650 655
Ser Phe Leu Ile Ile Thr Gly Pro Asn Met Gly Gly Lys Ser Thr Tyr
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675 680 685
Val Pro Cys Thr Glu Ala Glu Leu Thr Ile Phe Asp Cys Ile Leu Ala
690 695 700
Arg Val Gly Ala Ser Asp Ser Gln Leu Lys Gly Val Ser Thr Phe Met
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Ala Glu Met Leu Glu Thr Ser Asn Ile Leu Lys Ser Ala Thr Ser Glu
725 730 735
Ser Leu Ile Ile Ile Asp Glu Leu Gly Arg Gly Thr Ser Thr Tyr Asp
740 745 750
Gly Phe Gly Leu Ala Trp Ala Ile Ser Glu His Ile Val Thr Glu Ile
755 760 765
Arg Cys Phe Gly Leu Phe Ala Thr His Phe His Glu Leu Thr Ala Leu
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Ala Asp Arg Tyr Pro Lys Ser Val Lys Asn Leu His Val Val Ala Phe
785 790 795 800

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Ser Phe Gly Ile His Val Ala Glu Leu Val Arg Phe Pro Glu Lys Val
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    850                 855                 860
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865                 870                 875                 880
Gln Glu Glu Val Glu Glu Gly Ser Ala Leu Leu Lys Ala Met Leu Leu
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00063

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 1/15, C12N 15/10, C12P 9/00 // (C12N 1/15, C12R 1:66), (C12N 1/15, C12R 1:77)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9831837 A1 (MAXYGEN INC.), 23 July 1998 (23.07.98), see page 21, line 31 - page 23, line 20 and page 45, line 12 - page 56, line 21, esp. page 56, line 8 - line 12 --	1-15
A	Database SWISS-PROT, accession no. 013396, Huber D.H.: "DNA Mismatch Repair Protein MSH2. Neurospora crassa." 15 DEC 1998 --	1-15
A	WO 9705268 A1 (SETRATECH), 13 February 1997 (13.02.97), cited in the application --	1-15

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 June 2000	16 -06- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Hampus Rystedt/Els Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00063

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9737011 A1 (SETRATECH S.A.R.L.), 9 October 1997 (09.10.97), cited in the application --	1-15
A	WO 9007576 A1 (SETRATECH), 12 July 1990 (12.07.90), cited in the application -- --------	1-15

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/DK 00/00063

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9831837 A1	23/07/98	AU 5920998 A	07/08/98
WO 9705268 A1	13/02/97	AU 4784996 A	26/02/97
		EP 0842289 A	20/05/98
WO 9737011 A1	09/10/97	NONE	
WO 9007576 A1	12/07/90	AT 127519 T	15/09/95
		AU 4834390 A	01/08/90
		CA 2006549 A	26/06/90
		DE 68924174 D,T	14/03/96
		EP 0449923 A,B	09/10/91
		SE 0449923 T3	
		ES 2077058 T	16/11/95
		FR 2641793 A,B	20/07/90
		IE 72469 B	09/04/97
		IL 92856 D	00/00/00
		JP 4503601 T	02/07/92
		US 5912119 A	15/06/99
		US 5965415 A	12/10/99